

METHODS, SYSTEMS, AND USES FOR CALIXARENES

BACKGROUND OF THE INVENTION

[0001] The present invention relates to the general field of chemical sensing, and more particularly to calixarene compositions, devices and methods of use for identifying and detecting the presence of nitrogen oxide compounds in gas/vapor or liquid mixture.

5 [0002] Nitrogen oxide compounds, the so-called NO_x gases—a collective term that refers to the family of oxides of nitrogen—are toxic atmospheric pollutants derived generally from fossil fuel combustion, power plants, and large-scale industrial processes. NO_x are involved in the formation of ground-level ozone, participate in global warming, and also form toxic chemicals, nitrate particles and acid rain/aerosols. In addition, NO_x are active components in
10 various harmful nitrosation processes in biological tissues. Free radical NO rapidly reacts with oxygen, producing N_2O_3 (e.g., $\text{NO}\cdot\text{NO}_2$) and $\text{NO}_2/\text{N}_2\text{O}_4$ that are powerful nitrosating agents, both in the gas phase and in solution.

[0003] The pathophysiologic significance of NO_x derives from their ability to generate mutagenic nitrosopeptides and diazopeptides, to produce carcinogenic nitrosoamines, and to
15 nitrosate and deaminate DNA nucleobases. According to The United States Environmental Protection Agency, national emissions of NO_x have increased over the past 20 years by 4% and continue to rise. With the increase in NO_2 circulation in the atmosphere, there remains a need to not only systematically monitor nitrogen levels in the atmosphere, and to develop improved methods of fixation and utilization of nitrogen oxide compounds.

[0004] The present invention addresses many of the needs mentioned above as well as other objectives that will be appreciated by those skilled in the art.

SUMMARY OF THE INVENTION

5 [0005] The present invention solves many problems associated with current increases in NO_x levels in the atmosphere. The present invention provides new and improved methods of detecting and identifying of NO_x compounds, provides new and improved uses of these compounds as well as methods for storage of NO_x compounds. New and improved compounds for use in such applications are also provided.

10 [0006] In accordance with one aspect of the present invention, a method of and device for identifying, detecting and/or chemically utilizing NO_x compounds is provided, wherein simple calixarenes, such as calix[4]arenes (e.g., tetra-*O*-alkylated calix[4]arenes) are exposed to nitrogen oxides capable of producing nitrosonium ions (i.e. NO⁺) and form calixarene-nitrosonium complexes that are readily identified. Importantly, the process of forming one or more calixarene-nitrosonium complexes is reversible. In addition, the calixarene-
15 nitrosonium decolor with the addition of anything that reacts with the nitrosonium ion (e.g., water, alcohol) enabling the parent calixarenes (e.g., calix[4]arenes) to be recovered.

[0007] In accordance with another aspect of the present invention, a method of and device for attaching functionalized calix[4]arenes to a solid support (e.g., silica gel) is provided resulting in a solid material capable of identification, detection and/or entrapment of NO_x
20 compounds. As such nitrogen oxide compounds may be readily purified by means of the present invention.

[0008] Further, in accordance with the present invention, a method of and device for using calixarene-nitrosonium complexes is provided for the transfer of NO⁺ for nitrosation reactions. As such, novel nitrogen oxides storing materials are also provided.

25 [0009] An advantage of the present invention is that it provides a new and improved charge-transfer interaction for NO_x compounds (NO_x) allowing the compounds to be readily

detected in the presence of other gases, including H₂O, O₂, HCl, SO_x, NH₃, and even NO. In addition, the NO_x compounds and complexes formed with the present invention are vehicles for nitrosonium transfer and for encapsulated nitrosating reagents. The complexes also serve as nitrogen oxide (e.g., NO₂/NO_x) detection, sensing materials.

- 5 [00010] Those skilled in the art will further appreciate the above-mentioned advantages and superior features of the invention, together with other important aspects thereof upon reading the detailed description that follows in conjunction with the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

- 10 [00011] For a more complete understanding of the features and advantages of the present invention, reference is now made to the detailed description of the invention along with the accompanying FIGURES in which corresponding numerals in the different FIGURES refer to corresponding parts and in which:

FIGURE 1 depicts an example of chemical fixation of NO₂/N₂O₄ with calix[4]arene and formation of an encapsulated nitrosonium complex;

- 15 FIGURE 2 depicts two methods of preparing nitrosonium complexes of calix[4]arene compound 1 with (Left) addition of NO⁺SbF₆⁻ to compound 1 in chloroform, (Right) the reaction of NO₂/N₂O₄ with compound 1 in the presence of SnCl₄ in chloroform and (Below) MacroModel representation of an NO⁺ encapsulation process, wherein the CH hydrogen and long alkyl chains are omitted from view;

- 20 FIGURE 3 depicts two methods of synthesizing nitrosonium complexes with a MacroModel representation of an NO⁺ encapsulation process, wherein the CH hydrogen and long alkyl chains are omitted from view;

- 25 FIGURE 4 depicts an example of mesitylene-derived calixarene compound 6 that does not encapsulate NO⁺ showing a space-filling representation of compound 6 without the long alkyl chains;

FIGURE 5 depicts portions of the ^1H NMR spectra (500 MHz, CDCl_3 , 295 ± 1 K) of (A) calix[4]arene compound **1**, (B) nitrosonium complex **7**, wherein the identical spectrum was obtained upon addition of $\text{NO}^+\text{SbF}_6^-$ to compound **1**, (C) calix[4]arene compound **2** and (D) nitrosonium complex **8**, wherein the identical spectrum was obtained upon addition of $\text{NO}^+\text{SbF}_6^-$ to compound **2** and, wherein the residual CHCl_3 signals are marked with a dark circle;

FIGURE 6 depicts a host-guest dynamic in a calix[4]arene- NO^+ complex;

FIGURE 7 depicts NO^+ transfer between calixarene containers and formation of complex **9** from complex **8**;

FIGURE 8 depicts an example of a nitrosonium transfer experiment with portions of the UV-vis spectra;

FIGURE 9 depicts portions of the ^1H NMR spectra (500 MHz, CDCl_3 , 295 ± 1 K) of (A) nitrosonium complex **8**, obtained from compound **2**, NO_2 and SnCl_4 , (B) mixture of complex **8** and calixarene compound **5** after 20 minutes with about 30% conversion to complex **9**, (C) the same mixture after 1 hour, wherein complex **9** is formed with >95% conversion, and (D) nitrosonium complex $5 \cdot \text{NO}^+\text{SbF}_6^-$, independently obtained from compound **5** and $\text{NO}^+\text{SbF}_6^-$;

FIGURE 10 depicts a preparation of calix[4]arene functionalized silica gel **15** with (A) Et_3N , THF, rt, 12 h and an example of NO_2 entrapment chromatography experiment; and

FIGURE 11 depicts an *N*-Nitrosation reaction using secondary amides **16a-c** with complex **8** to form *N*-nitrosoamides **17a-c** and shows two prereactive complexes as examples.

DETAILED DESCRIPTION OF THE INVENTION

[00012] Although making and using various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many inventive concepts that may be embodied in a wide variety of contexts. The specific aspects
5 and embodiments discussed herein are merely illustrative of ways to make and use the invention, and do not limit the scope of the invention.

[00013] Nitrogen oxide compounds, also referred to as NO_x gases are toxic atmospheric compounds as well as pathophysiologic compounds. As used herein, NO_x refers to any mixture of oxides of nitrogen capable of generating a nitrosonium ion. One of the most
10 prevalent of these compounds is NO_2 , a paramagnetic gas since it has an unpaired electron on the nitrogen. It has an intense brown-orange color. When reacting with itself, it forms a colorless dimer, dinitrogen tetroxide (N_2O_4). For N_2O_4 , there is a very weak N-N bond and, at higher temperatures, the gas rapidly dissociates back to NO_2 . The position of equilibrium between the two compounds (N_2O_4 and NO_2) and the color of the system varies with
15 temperature. Below -21 degrees Centigrade, only pure, solid N_2O_4 is present. Above 140 degrees Centigrade the system is 100% NO_2 . This dynamic interconversion between NO_2 and N_2O_4 makes it impossible to study either of species alone. Of particular importance for mammals is that N_2O_4 may disproportionate to ionic NO^+NO_3^- .

[00014] Calix[4]arenes are building block-like molecules and have been used in the
20 construction of molecular containers (e.g., cavitands, (hemi)carcerands, and capsules). *Cone*-shaped calix[4]arenes are about 4 \AA deep and about 7 \AA in diameter at the upper rim. Tetra-*O*-alkylated *cone* calix[4]arenes exist in the pinched C_{2v} symmetrical conformation, with two opposite aromatic rings almost parallel and situated about 5 \AA apart, and two others flattened. This conformation is more preferable than the perfect cone C_{4v} conformation. Usually, the
25 interconversion between two C_{2v} structures is fast on the NMR time scale. Calix[4]arenes in a *1,3-alternate* conformation are much more rigid and possess a cylindrical inner tunnel, defined by two cofacial pairs of aromatic rings oriented orthogonal along the cavity axis. According to a number of X-ray studies, this tunnel is about $5\text{-}6 \text{ \AA}$ in diameter.

[00015] Complexes of calix[4]arenes with neutral molecules are weak. The cavities are obviously too small and they lack additional binding sites. In most of the crystal structures of the inclusion complexes of calixarenes, the guest molecule (i.e., the molecule that forms a complex with a calixarene) is positioned not inside, but roughly above the plane defined by the upper carbon atoms of the cyclic polyaromatic skeleton. Cations are known to interact with more strength with a calixarene π -surface. Ammonium ions and metal cations have been found to complex within the cone-shaped cavities. *1,3-Alternates*, functionalized with appropriate binding sites on the phenol oxygens, bind metal cations (e.g., Na^+ , K^+ , and Ag^+) both with “hard” oxygens and “soft” π -basic aromatic rings.

[00016] The present invention has found that interactions between calix[4]arenes and nitrogen oxide compounds leads to strong calixarene-nitrosonium complexes. FIGURE 1 shows the host-guest complexes formed upon interaction between NO_x and simple calix[4]arenes. In fact, calixarene complexes of the present invention are identified by: (1) an ability to react with NO_x to form stable nitrosonium (NO^+) complexes; (2) identification and detection (e.g., colorization) of the calixarene- NO^+ complexes; and (3) dissociation of the identified calixarene- NO^+ complexes (e.g., decoloration of the complex via anything that reacts with the nitrosonium ion, such as water or alcohol, as examples). Importantly, the calixarene- NO^+ complexes may be used for the transfer of NO^+ compounds, nitrosation reactions, NO_x identification, NO_x detection, and NO_x sensing, as well as serving as supramolecular NO^+ storing materials.

General Procedures

[00017] General methods of the present invention are described here and include those in Zyryanov G., et al. JACS. 2003; 125:2997-3007 (incorporated herein by reference). Melting Points were determined on a Mel-Temp apparatus (Laboratory Devices, Inc.) and a Buchi apparatus. ^1H and ^{13}C NMR spectra were recorded in CDCl_3 at $295 \pm$ degrees Centigrade, unless stated otherwise, on JEOL Eclipse 500 MHz spectrometer. Chemical shifts were measured relative to residual non-deuterated solvent resonances. FTIR spectra were recorded on a Bruker Vector 22 FTIR spectrometer. UV-vis spectra were measured on a JASCO V-

530 spectrophotometer. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF) was performed on a Bruker BiFLEX I linear time-of-flight mass spectrometer operated in delayed extraction mode. Elemental analysis was performed on a Perkin-Elmer 2400 CHN analyzer. For column chromatography, Silica Gel 60 Å (Sorbent Technologies, Inc.; 200–425 mesh) was used. All experiments with moisture- or air-sensitive compounds were run in freshly distilled, anhydrous solvents under a dried nitrogen atmosphere. Molecular modeling was performed generally using MacroModel 7.1.

[00018] Parent tetrahydroxycalix[4]arenes were prepared according to procedures known to one of ordinary skill in the art (see e.g., Gutsche, C. D., Iqbal, M. *Org. Synth.* 1990, Vol. 68, pp. 234-237). NO₂/N₂O₄ was generated from copper and concentrated HNO₃ or sodium nitrate and concentrated H₂SO₄.

Example for Preparing 25,26,27,28-Tetra (*n*-hexyloxy)calix[4]arene-1,3-alternate (compound 2).

[00019] To a suspension of 25,27,26,28-tetrahydroxycalix[4]arene (about 4.24 g, 0.01 mol) and K₂CO₃ (about 4.2 g, 0.03 mol) in MeCN (about 200 mL) *n*-hexylbromide (about 4.2 mL, 0.03 mol) was added, and the reaction mixture was refluxed under nitrogen for about 48 hours. The precipitate was filtered off, and the solution evaporated to dryness. The residue was redissolved in CH₂Cl₂ (about 200 mL), and the solution was washed with water (3 x 150 mL) and dried over MgSO₄. After evaporation, the solid residue was treated with MeOH (about 200 mL) to yield pure 25,27-bis(*n*-hexyloxy)-26,28-hydroxycalix[4]arene as a white solid (5.0 g, 84%). ¹H NMR results: δ 8.23 (bs, 2 H), 7.06 (d, *J* = 8 Hz, 4 H), 6.91 (d, *J* = 8 Hz, 4 H), 6.73 (t, *J* = 8 Hz, 2 H), 6.65 (t, *J* = 8 Hz, 2 H), 4.32 (d, *J* = 13 Hz, 4 H), 4.00 (t, *J* = 7 Hz, 4 H), 3.37 (d, *J* = 13 Hz, 4 H), 2.1-2.0 (m, 4 H), 1.75-1.7 (m, 4 H), 1.45-1.4 (m, 8 H), 0.95 (t, *J* = 7 Hz, 6 H).

[00020] To a suspension of 25,27-bis(*n*-hexyloxy)-26,28-hydroxycalix[4]arene (about 5.92 g, 0.01 mol) and Cs₂CO₃ (about 50 g, 0.15 mol) in MeCN (about 300 mL) *n*-hexylbromide (about 5.74 mL, 0.04 mol) was added, and the reaction mixture was refluxed under nitrogen for about 48 hours. After cooling, the precipitate was filtered off and treated with a mixture

of water (about 100 mL) and CH₂Cl₂ (about 100 mL). The organic layer was separated, washed with water (2 x 100 mL), dried over MgSO₄, and evaporated. The residue was recrystallized from MeOH-CHCl₃, 10:1 to give pure compound **2** (3.50 g, 46%). Melting Point: 119 degrees Centigrade. ¹H NMR results were: δ 6.92 (d, *J* = 7.5 Hz, 8 H), 6.68 (t, *J* = 7.5 Hz, 4 H), 3.62 (s, 8 H), 3.54 (t, *J* = 7.5 Hz, 8 H), 1.89 (m, 8 H), 1.35 (m, 24 H), 0.85 (t, *J* = 7 Hz, 12 H). MALDI-TOF MS, *m/z* 783.9 ([M+Na⁺], calculated for C₅₂H₇₂O₄ 783.9).
Anal. calculated for C₅₂H₇₂O₄: C, 82.06; H, 9.53. Found: C, 81.61; H, 9.58.

Example for Preparing 5,11,17,23-Tetrabromo-25,26,27,28-tetrakis(*n*-hexyloxy)calix[4]arene-1,3-alternate (compound **3**).

10 [00021] *N*-Bromosuccinimide (about 3.0 g, 0.017 mol) was added to a suspension of calixarene **2** (about 2.0 g, 2.6 mmol) in acetone (about 500 mL), and the mixture was stirred at room temperature for about 48 hours and exposed to the laboratory light. The formed solid was filtered off, washed with acetone (about 2 x 100 mL) and used in the next step without further purification. Yield 1.5 g (53 %). ¹H NMR results were: δ 7.12 (s, 8 H), 3.56 (s, 8 H),
15 3.52 (t, *J* = 7.5 Hz, 8 H), 1.6-1.4 (m, 8 H), 1.4-1.3 (m, 24 H), 0.93 (t, *J* = 7 Hz, 12 H).

Example for Preparing 5,11,17,23-Tethydroxy-25,26,27,28-tetrakis(*n*-hexyloxy)calix[4]arene-1,3-alternate (compound **4**).

[00022] To a solution of tetrabromo derivative compound **3** (about 0.9 g, 0.83 mmol) in freshly distilled over Na and oxygen-free THF (about 150 mL) *n*-BuLi (about 30 mL of 2M solution in pentane, 60 mmol) was added at -78 degrees Centigrade, and the mixture was
20 stirred at this temperature for about 75 minutes. Trimethyl borate (about 14 mL, 145 mmol) was then added at -78 degrees Centigrade, and the mixture was allowed to warm to room temperature. After about 5 hours, the reaction mixture was cooled down again to -78 degrees Centigrade, and H₂O₂ (about 15 mL of 30% aqueous solution) and NaOH (about 35
25 mL of 3N aqueous solution) was added. The resulting solution was stirred overnight at room temperature, after which the precipitate was filtered off. The mother liquor was cooled to 0 degrees Centigrade and treated with NaS₂O₃ (about 25 g), after which the mixture was filtered and the filtrate was concentrated in vacuo. The residue was treated with about 5% aqueous HCl (100 mL), and the colorless precipitate of compound **4** was filtered off and

washed with MeOH (about 50 mL). Yield 0.274 g (40%). Melting Point: 230 degrees Centigrade. ^1H NMR results were (DMSO- d_6): δ 8.39 (bs, 4 H), 6.34 (s, 8 H), 3.50 (s, 8 H), 3.02 (t, J = 7.5 Hz, 8 H), 1.3-1.25 (m, 8 H), 1.25-1.1 (m, 16 H), 1.1-1.0 (m, 8 H), 0.87 (t, J = 7 Hz, 12 H). MALDI-TOF MS, m/z 847 ($[\text{M}+\text{Na}^+]$, calculated for $\text{C}_{52}\text{H}_{72}\text{O}_8$ 847).

- 5 Example for Preparing 5,11,17,23,25,26,27,28-Octa(*n*-hexyloxy)calix[4]arene-1,3-alternate (compound 5).

[00023] To a suspension of compound 4 (about 0.3 g, 0.36 mmol) and NaH [about 0.15 g of about 60% (wt.) suspension in mineral oil, 3.6 mmol] in freshly distilled DMF (about 50 mL) *n*-hexylbromide (about 0.46 mL, 3.2 mmol) was added, and the reaction mixture was
10 stirred at about 70 degrees Centigrade under nitrogen for about 24 hours. The precipitate was filtered off, and the mother liquor was treated with a mixture of crushed ice (about 50 g), water (about 50 mL) and CH_2Cl_2 (about 100 mL). The organic layer was separated, washed with water (2 x 100 mL), dried over MgSO_4 and evaporated. The residue was recrystallized from MeOH- CHCl_3 , 10:1 to yield calixarene compound 5 (0.35 g, 85 %). Melting Point: 11
15 degrees Centigrade. ^1H NMR results were: δ 6.55 (s, 8 H), 3.83 (t, J = 7 Hz, 8 H), 3.62 (s, 8 H), 3.35 (t, J = 7 Hz, 8 H), 1.7 (m, 16 H), 1.4-1.2 (m, 48 H), 0.89 (t, J = 7 Hz, 24 H). ^{13}C NMR results were: δ 153.6, 150.7, 134.4, 115.2, 71.6, 68.1, 37.8, 32.3, 31.8, 29.8, 26.0, 25.9, 22.9, 22.7, 14.3, 14.1; MALDI-TOF MS, m/z 1182 ($[\text{M}+\text{Na}^+]$, calculated for $\text{C}_{76}\text{H}_{120}\text{O}_8$ 1183).

- 20 Example for Preparing Tetrakis(*O*-*n*-hexyloxy)cyclophane (compound 6).

[00024] NaH (about 0.11 g of about 60% suspension in mineral oil, 2.7 mmol) was added to the solution of Pappalardo's cyclophane (about 0.2 g, 0.34 mmol prepared using methods known in the art) in freshly distilled DMF (about 20 mL), and the mixture was stirred under nitrogen for about 30 minutes. *n*-Hexylbromide (about 0.3 mL, 2.04 mmol) was then added,
25 and the reaction mixture was stirred at about 70 degrees Centigrade for 3 days. The precipitate was collected and dissolved in CH_2Cl_2 (about 20 mL). The solution was washed with water (3 x 20 mL), dried over MgSO_4 and evaporated. The residue was recrystallised from MeOH- CHCl_3 to afford compound 6 as a white powder. Yield 0.157 g (50 %). ^1H

NMR results were: δ 3.89 (s, 8 H), 3.58 (t, $J = 7$ Hz, 8 H), 2.30 (s, 24 H), 1.8-1.7 (m, 8 H), 1.6-1.5 (m, 16 H), 1.4-1.35 (m, 8 H), 1.07 (s, 12 H), 0.91 (t, $J = 7$ Hz, 12 H). ^{13}C NMR results were: δ 153.9, 138.4, 131.4, 126.7, 73.2, 32.8, 32.0, 30.4, 26.0, 22.8, 17.7, 14.2, 13.7. Anal. calculated for $\text{C}_{64}\text{H}_{96}\text{O}_4$: C 82.70; H 10.41. Found: C, 82.37; H, 10.25.

5 Example for Preparing Calix[4]arene-nitrosonium Complexes.

[00025] In one example, stock solutions of NO_2 were freshly prepared upon bubbling through CHCl_3 ; the gas concentration was determined gravimetrically. In a typical procedure, a solution of calixarene compound 1 (about 1 equivalent) in dry, freshly distilled CHCl_3 was mixed with the stock solution of NO_2 (about 3 equivalent) in CHCl_3 and SnCl_4 (about 1.5 eq) at room temperature. After 1 hour, complex 7 was precipitated upon addition of hexane, filtered off, washed with hexane (about 2 times), and dried in vacuo. ^1H NMR results were: δ 6.99 (s, 8 H), 4.39 (d, $J = 13$ Hz, 4 H), 4.02 (t, $J = 7.5$ Hz, 8 H), 3.44 (d, $J = 13$ Hz, 4 H), 2.0-1.9 (m, 8 H), 1.5-1.3 (m, 24 H), 0.93 (t, $J = 7$ Hz, 12 H); UV-vis (CHCl_3): λ_{max} 563; FTIR (CDCl_3): ν 1923 (NO^+), 1461, 1298, 1047 (NO_3^-); Anal. calculated for $\text{C}_{68}\text{H}_{104}\text{O}_4 \cdot \text{NO}^+ \text{NO}_3^- \cdot 1.8\text{SnCl}_4$: C, 52.81; N, 1.81; H, 6.78. Found: C, 52.60; N, 1.67; H, 7.55. Complex 8 was obtained analogously and ^1H NMR results were: δ 7.17 (t, $J = 7.5$ Hz, 4 H), 7.08 (d, $J = 7.5$ Hz, 8 H), 3.87 (t, $J = 7.5$ Hz, 8 H), 3.60 (s, 8 H), 1.9-1.8 (m, 8 H), 1.4-1.3 (m, 24 H), 0.93 (t, $J = 7$ Hz, 12 H); UV-vis (CHCl_3): λ_{max} 524 nm; FTIR (CDCl_3): ν 1955 (NO^+), 1438, 1246 1901, (NO_3^-); Anal. calculated for $\text{C}_{52}\text{H}_{72}\text{O}_4 \cdot \text{NO}^+ \text{NO}_3^- \cdot 1.5\text{SnCl}_4$: C, 50.21; N, 2.25; H, 5.83. Found: C, 50.23; N, 1.82; H, 5.99. Complex 9 is generally not prepared using this protocol; because, only dealkylation/oxidation products were detected. For spectroscopic characterization, complex 9 was generated in the exchange experiment between nitrosonium complex 8 and free calixarene compound 5, as depicted in FIGURE 7.

[00026] In another example, calix[4]arene-nitrosonium complexes were obtained upon mixing compound 1, 2 or 5 with an excess $\text{NO}^+ \text{SbF}_6^-$ in dry CHCl_3 . Complexes $1 \cdot \text{NO}^+ \text{SbF}_6^-$ and $2 \cdot \text{NO}^+ \text{SbF}_6^-$ formed within about 20 hours. The UV-vis, FTIR and ^1H NMR spectra were identical with the respective complexes 7 and 8. Complex $5 \cdot \text{NO}^+ \text{SbF}_6^-$ formed immediately upon mixing. ^1H NMR results were: δ 6.54 (s, 8 H), 3.93 (t, $J = 7.5$ Hz, 8 H), 3.76 (t, $J = 7.5$

Hz, 8 H), 3.44 (s, 8 H), 1.8-1.75 (m, 8 H), 1.9-1.8 (m, 8 H), 1.5-1.2 (m, 48 H), 0.95 (t, $J = 7$ Hz, 12 H), 0.89 (t, $J = 7.5$ Hz, 12 H); UV-vis (CHCl_3): λ_{max} 600; FTIR (CDCl_3): $\nu(\text{NO}^+)$ 1876.

5 Example for Preparing 25-Hydroxy-26,27,28-trihexyloxy-*p*-*tert*-butylcalix[4]arene (compound 10).

[00027] A mixture of 25,26,27,28-tetrahydroxy-*p*-*tert*-butylcalix[4]arene (about 4.0g, 6.2 mmol), freshly distilled anhydrous DMF (about 80 mL), $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (about 6.8g, 21.6 mmol), and BaO (about 6.36g, 41.5 mmol) were stirred at room temperature for 15 minutes. *n*-Hexylbromide (about 21 mL, 184 mmol) was added, and the suspension was stirred at
10 room temperature for another about 12 hours. The mixture was diluted with water (about 100 mL), and the product was extracted with CH_2Cl_2 (3 x 100 mL). The organic layer was washed with water (2 x 100 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The residue was recrystallized from MeOH to give pure compound 10 as a white solid. Yield 69%; Melting Point: 134-136 degrees Centigrade. ^1H NMR results were: δ 7.11 (s, 2 H),
15 7.03(s, 2 H), 6.51 (d, $J = 2.3$ Hz, 2 H), 6.49 (d, $J = 2.3$ Hz, 2 H), 5.72 (s, 1 H), 4.36 (d, $J = 13$ Hz, 2 H), 4.32 (d, $J = 13$ Hz, 2 H), 3.89 (t, $J = 8$ Hz, 2 H), 3.78 (t, $J = 8$ Hz, 4 H), 3.22 (d, $J = 13$ Hz, 2 H), 3.16 (d, $J = 13$ Hz, 2 H), 2.3-2.2 (m, 2 H), 2.0-1.8 (m, 4 H), 1.4-1.3 (m, 18 H), 1.32 (s, 9 H), 1.31 (s, 9 H), 0.92 (t, $J = 7$ Hz, 3H), 0.90 (m, 6 H), 0.81 (s, 18 H).

20 Example for Preparing 25-[(Ethoxycarbonyl)methoxy]-26,27,28-trihexyloxy-*p*-*tert*-butylcalix[4]arene (11).

[00028] A mixture of calix[4]arene compound 10 (about 5.0g, 5.6 mmol) and Na_2CO_3 (about 10.0g, 94 mmol) in CH_3CN (about 150 mL) was refluxed for about 15 minutes, after which ethyl bromoacetate (about 10 mL, 90 mmol) was added, and the reflux continued for 12 hours. The inorganic salts were filtered, and the solvent was evaporated. The residue was
25 dissolved in CH_2Cl_2 (about 100mL) and washed with water (3 x 50 mL). The solvent was evaporated, and the product was recrystallized from MeOH. Yield 89%; Melting Point: 121-123 degrees Centigrade. ^1H NMR results were: δ 6.91(s, 2 H), 6.90 (s, 2 H), 6.63 (d, $J = 2$ Hz, 2 H), 6.61 (d, $J = 2$ Hz, 2 H), 4.86 (s, 2 H), 4.66 (d, $J = 12.5$ Hz, 2 H), 4.38 (d, $J = 12.5$

Hz, 2 H), 4.18 (q, $J = 7$ Hz, 2 H), 3.83 (t, $J = 8$ Hz, 4 H), 3.75 (t, $J = 8$ Hz, 2 H), 3.15 (d, $J = 12.5$ Hz, 2 H), 3.10 (d, $J = 12.5$ Hz, 2 H), 2.2-2.1 (m, 2 H), 2.0-1.9 (m, 4 H), 1.4-1.3 (m, 18 H), 1.27 (t, $J = 7$ Hz, 3 H), 1.18 (s, 9 H), 1.17 (s, 9 H), 0.96 (s, 18 H), 0.9-0.8 (m, 9 H).

5 Example for Preparing 25-(Carbomethoxy)-26,27,28-trihexyloxy-*p*-*tert*-butylcalix[4]arene (compound 12).

[00029] A mixture of compound 11 (about 2.0 g 2 mmol), THF-H₂O, about 5:1 (100 mL) and KOH (about 1.0 g, 17.8 mmol) was refluxed for about 12 hours. The pH was adjusted to about 4 with aqueous 2 M HCl. The product was extracted with CH₂Cl₂ (2 x 50 mL), and the organic layer was dried over Na₂SO₄ and evaporated to give compound 12 as a white solid.

10 Yield >95%; Melting Point: 136-137 degrees Centigrade. ¹H NMR results were: δ 11.28 (s, 1 H), 7.16 (s, 2 H), 7.14 (s, 2 H), 6.59 (d, $J = 2.5$ Hz, 2 H), 6.49 (d, $J = 2.5$ Hz, 2 H), 4.67 (s, 2 H), 4.45 (d, $J = 12$ Hz, 2 H), 4.23 (d, $J = 12$ Hz, 2 H), 4.08 (t, $J = 7$ Hz, 2 H), 3.8-3.7 (m, 4H), 3.24 (d, $J = 12$ Hz, 2 H), 3.16 (d, $J = 12$ Hz, 2 H), 1.95-1.8 (m, 6 H), 1.45-1.2 (m, 18 H), 0.90 (t, $J = 7$ Hz, 9 H), 0.83 (s, 18 H); MALDI-TOF MS, m/z 959.2 (M^+ , calculated for
15 C₆₄H₉₄O₆ 960.4). Anal. calculated for C₆₄H₉₄O₆: C 80.12; H 9.88. Found: C, 80.27; H, 9.88.

Example for Preparing *N*-Succinimide Ester of 25-(Carbomethoxy)-26,27,28-trihexyloxy-*p*-*tert*-butylcalix[4]arene (compound 13).

[00030] A suspension of compound 12 (about 1.0g, 1.05 mmol), *N*-hydroxy succinimide (about 1.0g, 8.70 mmol), DCC (210 mg 1.05 mmol) and DMAP (about 40 mg, 0.32 mmol) in
20 THF (about 50 mL) was stirred at room temperature for about 12 hours under nitrogen. After filtration, the solvent was evaporated, and the residue was redissolved in hexane (50 mL) and filtered again. The hexane solution was evaporated and the residue was purified by column chromatography (about CH₂Cl₂-hexane, 1:1) to afford compound 13 as a white solid. Yield 83%; Melting Point: 75-78 degrees Centigrade; ¹H NMR results were: δ 6.95 (s, 2 H), 6.92
25 (s, 2 H), 6.62 (d, $J = 2.5$ Hz, 2 H), 6.56 (d, $J = 2.5$ Hz, 2 H), 5.28 (s, 2 H), 4.56 (d, $J = 13$ Hz, 2 H), 4.41 (d, $J = 13$ Hz, 2 H), 3.89 (t, $J = 8$ Hz, 2 H), 3.79 (t, $J = 8$ Hz, 2 H), 3.75 (t, $J = 8$ Hz, 2 H), 3.18 (d, $J = 13$ Hz, 2 H), 3.12 (d, $J = 13$ Hz, 2 H), 2.82 (s, 4 H), 2.15-2.1 (m, 2 H), 1.95-1.85 (m, 4 H), 1.45-1.35 (m, 18 H), 1.20, 1.19 (2 x s, 18 H), 0.93 (s, 18 H), 0.9-0.8 (m, 9 H). Ester compound 13 appeared to be relatively unstable; attempts to obtain the MALDI-

TOF and/or CHN analytical data failed. The structure of compound **13** was confirmed through its transformation to calixarene amide compound **14**.

Example for Preparing 25-[(*n*-Octylcarbamoylmethoxy)-26,27,28-trihexyloxy-*p*-*tert*-butylcalix[4]arene (compound **14**).

- 5 [00031] To the solution of ester compound **13** (about 75 mg, 70 μ mol) in THF (about 10 mL), *n*-octylamine (about 18 mg, 0.14 mmol) and Et₃N (about 70 mg, 0.7mmol) were added, and the reaction mixture was stirred at room temperature for about 12 hours. The precipitate was filtered off, and the solution was evaporated. The residue was redissolved in CH₂Cl₂ (about 20 mL), washed with 2 M aqueous HCl (2 x 5 mL) and water (about 5 mL), and dried
10 over Na₂SO₄. The organic layer was then evaporated and the residue was recrystallized from CH₃CN to afford compound **14** as a white solid. Yield 65%. ¹H NMR results were: δ 8.43 (t, *J* = 6 Hz, 1 H), 6.99 (s, 2 H), 6.97 (s, 2 H), 6.57 (s, 4 H), 4.71 (s, 2 H), 4.38, 4.35 (2 x d, *J* = 13 Hz, 4 H), 3.9-3.7 (2 x m, 6 H), 3.42 (dt, *J* = 6 Hz, *J* = 7 Hz, 2 H), 3.23 (d, *J* = 13 Hz, 2 H), 3.14 (d, *J* = 13 Hz, 2 H), 2.0-1.8 (3 x m, 8 H), 1.5-1.3 (m, 30 H), 1.25 (s, 9 H), 1.23 (s, 9 H),
15 0.93 (s, 18 H), 0.95-0.85 (m, 12 H). FTIR (KBr): ν 3346 (NH), 2964, 1680 (C=O), 1537, 1473. Anal. calculated for C₇₂H₁₁₁NO₅•0.5CH₃CN: C, 80.35; H, 10.39; N, 1.93. Found: C 80.02; H 10.09; N, 1.91. Compound **14** was also independently synthesized from the acid chloride of compound **12** (prepared with SOCl₂), *n*-octylamine and Et₃N in CHCl₃.

Example for Preparing a Calix[4]arene Functionalized Silica Gel (**15**).

- 20 [00032] A suspension of ester compound **13** (about 100 mg, 95 μ mol), 3- aminopropyl-functionalized silica gel (Aldrich) (about 226 mg, 155 μ mol) and Et₃N (about 92.9 mg, 0.92 mmol) in THF (50 mL) was stirred at room temperature for about 12 hours. The solid was filtered off, washed with CH₂Cl₂, MeOH, water, MeCN, and THF, and then dried under reduced pressure for about 3 days to provide a white powder. FTIR (KBr): ν 3383, 2960,
25 1650, 1556, 1477. Anal. Found for 3-aminopropyl silica gel (0.687 meq/g, C₉H₂₃NO₃Si): C, 7.42; H, 1.80; N, 1.91. Anal. Calcd for silica gel **15** (17% loading, 0.117 meq/g, C₇₃H₁₁₅NO₈Si): C, 15.43; H, 2.68; N, 1.82. Found: C 15.34; H 2.97; N, 1.70.

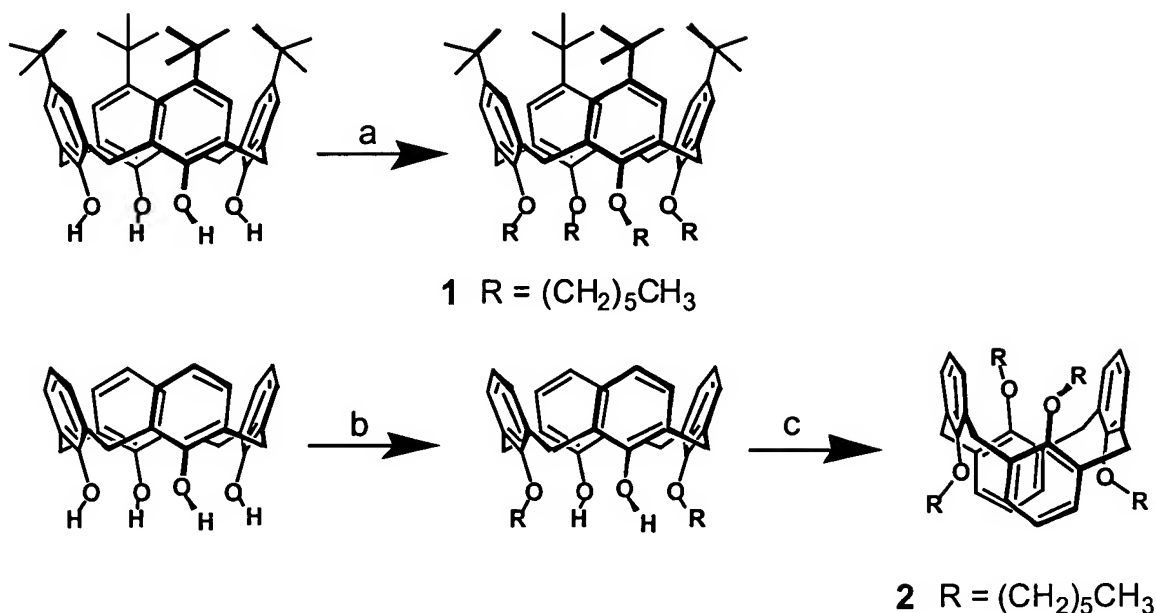
Example for Preparing *N*-Nitrosation of Amides by Calix[4]arene-Nitrosonium Complexes.
[It is noted that *N*-Nitrosoamides are potential carcinogens and should be treated with extreme care.]

[00033] Complex 8 (about 1 equivalent) was added to the solution of amide
5 AlkC(O)NHMe 16a-c (about 2-3 equivalents) in freshly distilled CHCl₃, and the reaction mixture was stirred at room temperature for about 5 hours. The solvent was evaporated, and the residue was analyzed by ¹H NMR spectroscopy and further separated by preparative TLC. The spectral data for the obtained *N*-nitroso compounds AlkC(O)N(NO)Me 17a-c were identical with those independently obtained from AlkC(O)NHMe and NO₂/N₂O₄ or
10 NO⁺SbF₆⁻, and similar to results observed by others. (See, e.g., Garcia, J., et al. J. Org. Chem. 1984, 49, pp. 3322-3327) For CH₃(CH₂)₆C(O)NHCH₃ (16b), ¹H NMR results were: δ 6.13 (bs, 1 H, NH), 2.73 (d, *J* = 5 Hz, 3 H, N-CH₃), 2.13 (t, *J* = 7.5 Hz, 2 H, C(O)CH₂), 1.6-1.5 (m, 2 H, CH₂), 1.3-1.1 (m, 8 H, CH₂), 0.83 (t, *J* = 7.5 Hz, 3 H, CH₃). For CH₃(CH₂)₆C(O)N(NO)CH₃ (17b), ¹H NMR results were: δ 3.13 (t, *J* = 8 Hz, 2 H, C(O)CH₂),
15 3.03 (s, 3 H, N(NO)-CH₃), 1.8-1.7 (m, 2 H, CH₂), 1.4-1.2 (m, 8 H, CH₂), 0.84 (t, *J* = 7.5 Hz, 3 H, CH₃).

General Procedures for Synthesis of Compounds and Complexes of the Present Invention.

[00034] Calix[4]arenes compounds 1 and 2, possessing a *cone* and a *1,3-alternate* conformation, respectively, were synthesized through *O*-alkylation of the corresponding
20 parent calix[4]arenes with *n*-hexylbromide (see SCHEME 1). In the synthesis of compound 1, NaH was employed as a base in hot DMF. The preparation of compound 2 may include the two-step alkylation of de-*t*-butylated calix[4]arene with *n*-hexyl bromide, using successively K₂CO₃ and then Cs₂CO₃ in boiling MeCN.

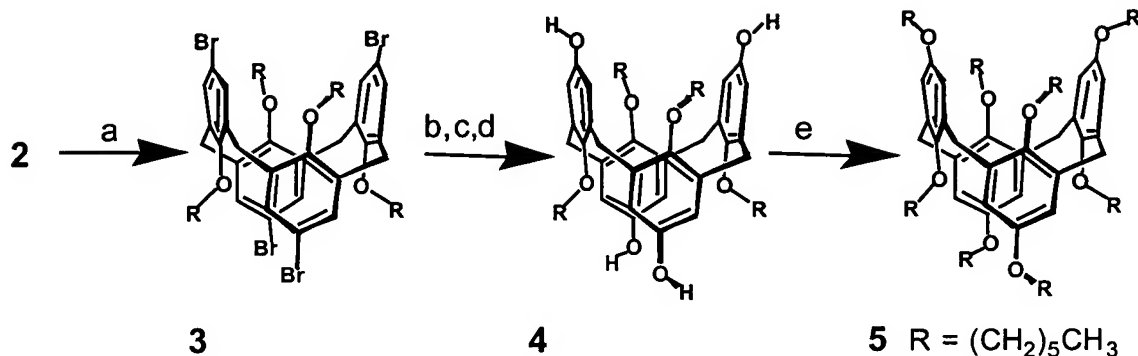
SCHEME 1



(a) *n*-hexyl bromide, NaH, DMF, 70 degrees Centigrade, 24 hours, 75% yield; (b) *n*-hexyl bromide, K_2CO_3 , MeCN, 80 degrees Centigrade, 48 hours, 84% yield; (c) *n*-hexyl bromide, Cs_2CO_3 , MeCN, 80 degrees Centigrade, 48 hours, 46% yield.

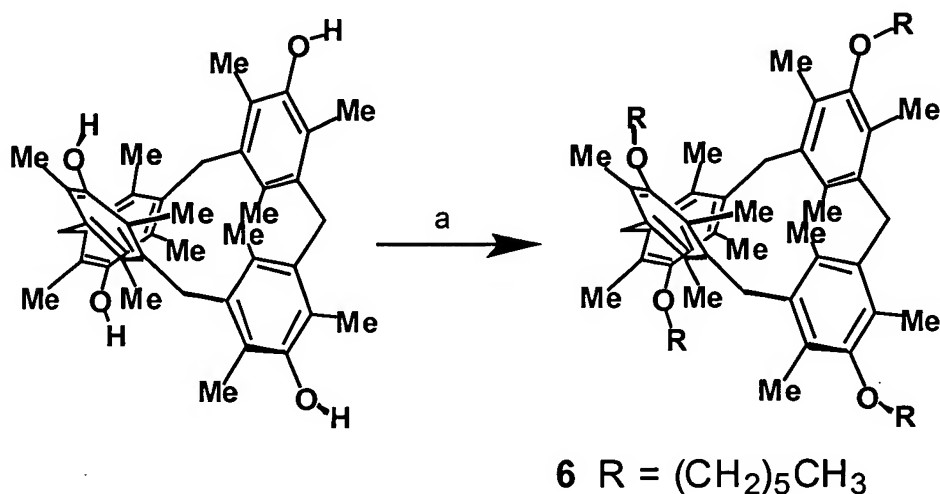
[00035] Bromination of compound 2 with NBS in acetone provides tetrabromocalix[4]arene compound 3 in a 53% yield (SCHEME 2). Tetrahydroxylated 1,3-alternate derivative compound 4 was obtained through bromo-lithium exchange in compound 3 (*n*-BuLi, THF, at -78 degrees Centigrade), followed by treatment with $B(OMe)_3$ and oxidation with H_2O_2 and aqueous NaOH (with a 40% yield after three steps). Calixarene compound 4 was subsequently alkylated with *n*-hexylbromide and NaH in hot DMF to yield octahexyloxycalix[4]arene compound 5 (about 85% yield). Mesitylene derived 1,3-alternate compound 6 was obtained for comparison, according to known procedures (see e.g., Pappalardo, S et al. *J. Org. Chem.* 1992, 57, pp. 7102-7109 or Staffilani, M. et al. *Organometallics* 1998, 17, pp. 1732-1740) and subsequent O-alkylation with *n*-hexylbromide (SCHEME 3).

SCHEME 2



(a) NBS, acetone, room temperature, 48 hours, 53% yield; (b) *n*-BuLi (60 eq), THF, -78 degrees Centigrade, 0.5 hours; (c) B(OMe)₃, -78 to 0 degrees Centigrade; (d) H₂O₂, aqueous NaOH, -78 to 25 degrees Centigrade, 40% yield (three steps); (e) *n*-hexyl bromide, NaH, DMF, 70 degrees Centigrade, 24 hours, 85% yield.

SCHEME 3



(a) *n*-hexyl bromide, NaH, DMF, 70 degrees Centigrade, 72 hours, 50% yield.

10 Interaction of Calix[4]arenes with Nitrogen Oxide Compounds of the Present Invention.

[00036] Bubbling NO₂ through the solutions of compounds 1, 2 and 5 in CHCl₃ resulted in instant identification of the complex, e.g., deep coloration. For example, solutions of compound 1 and compound 5 turned blue to dark blue, and a solution of compound 2 became purple to deep purple. The UV-vis spectra changed accordingly: the broad bands appeared at

15 λ_{max} of about 560, 512 and 600 nm for NO₂-exposed solutions of compounds 1, 2 and 5,

respectively. This is in contrast to the colorless solution of compounds 1, 2 and 5 and the yellow to pale yellow solution of NO₂ in CHCl₃ prior to mixing.

[00037] In the reactions with non-cyclic anisole (e.g., methoxybenzene), identification (e.g., coloration) occurred but was less pronounced upon exposure to NO₂. Moreover, when
5 mesitylene-derived, Pappalardo's calixarene 6, with the sterically *blocked* and conformationally much more *rigid* cavity, was tested, the complex was not detected (e.g., no coloration was observed). In compound 6, the pair of methyl groups in the *ortho*-positions relative to the oxygen forces the methyl groups of the adjacent aromatic rings towards each other, not only blocking an access to the cylindrical inner cavity, but also significantly
10 rigidifying it. The same effect takes place on the other side of the calixarene 6, which makes its interior completely blocked. These reactions show the importance of *cavities* in the described transformations and illustrate NO⁺ coordination inside 1, 2 and 5. (FIGURES 2, 3, and 4)

[00038] According to the molecular modeling performed on these compounds (e.g., using
15 MacroModel 7.1), one NO⁺ cation can fit inside the cavities of compounds 1, 2 and 5, and neither bent NO₂ nor bulky NO₃⁻ can be accommodated. Two parallel aromatic rings of a *cone* calix[4]arene participate in the NO⁺ complexation, and all four rings of a *1,3-alternate* calixarene are involved. In mesitylene derived calixarene 6, no NO⁺ encapsulation may occur for steric reasons.

[00039] While an instant detection of a complex (e.g., nitrosated compounds 1, 2, and 5 via
20 rapid coloration) shows charge-transfer between the prereactive nitrosating/nitrating species and the compounds (i.e., 1, 2, and 5), the actual charge-transfer itself may be difficult to monitor. Interaction of NO₂ with compounds 1, 2, and 5 is very dynamic, and initial ¹H NMR analysis of the solutions showed complex, quickly changing pictures; the NO₂/N₂O₄
25 mixture is an effective nitrosating/nitrating agent. Not surprisingly, the NO₂-containing CHCl₃ solutions of compounds 1 and 2 decolorized within or at about 1 to 2 hours, yielding mixtures of known *p*-nitrated calixarenes (preparative TLC, ¹H NMR). Calixarene 5, much more activated for the electrophilic aromatic substitution, reacted with NO₂ more quickly—

as quickly as within a few minutes—producing a complex mixture of dealkylated and oxidized products.

[00040] When compounds **1** and **2** are simultaneously treated with SnCl_4 and 2 to 3 equivalents of NO_2 , no nitration products are detected. Precipitation with hexanes resulted in detectable solids (e.g., deeply colored, moisture sensitive), assigned to nitrosonium complexes **7** and **8** (>90% yield, see FIGURES 2, 3, and 4). These complexes are very stable and can be stored, in the absence of moisture, for about several weeks, both in CHCl_3 solution and in the solid state. In one embodiment, complex **7** and **8** are identified by their color, wherein complex **7** is generally a dark blue and complex **8** is generally a deep purple.

10 Analysis and Characterization of Compounds and Complexes of the Present Invention.

[00041] Some spectral features of the obtained nitrosonium complexes are presented in the TABLE. The UV-vis spectra showed broad charge-transfer bands at λ_{max} of about 563 and 524 nm, and the FTIR spectra exhibited characteristic arene- NO^+ stretching at wavelengths of about $\nu = 1923 \text{ cm}^{-1}$ and 1955 cm^{-1} for complexes **7** and **8**, respectively. The ^1H NMR spectra of complexes **7** and **8** exhibited new sets of the calixarene signals as well. (FIGURE 5) In particular, aromatic CH protons of guest-free compound **1** were seen as a singlet at 6.76 ppm. In nitrosonium complex **7**, these were transformed into a singlet at 6.99 ppm. The methylene bridge CH_2 protons of compound **1** were recorded as doublets at 4.41 and 3.12 ppm ($J = 12.5 \text{ Hz}$). In complex **7**, these were seen as doublets at 4.39 and 3.44 ppm ($J = 13$ Hz). The aromatic protons of free compound **2** were seen as a doublet and a triplet, 2:1, at 6.92 and 6.68 ppm, respectively ($J = 7.5 \text{ Hz}$). In nitrosonium complex **8**, these were transformed into a triplet and a doublet, 1:2, at 7.17 and 7.08 ppm, respectively ($J = 7.5 \text{ Hz}$). The methylene bridge CH_2 and OCH_2 protons of compound **2** were seen as a singlet and a triplet, 1:1, at 3.62 and 3.54 ($J = 7.5 \text{ Hz}$), respectively. In complex **8**, these were transformed into a singlet and a triplet, 1:1, at 3.60 and 3.87 ($J = 7.5 \text{ Hz}$), respectively. Elemental analysis of the moisture sensitive and thermally unstable complexes **7** and **8** proved to be difficult but reproducibly showed the CHN ratios corresponding to the presence of only one NO^+ cation in both structures.

Table 1. Spectroscopic data for calix[4]arenes 1, 2, and 5, and their nitrosonium complexes.

Compound	δ , ppm	ν , cm^{-1}	λ_{max} , nm
1	6.76 (s, arom), 4.41, 3.12 (2 x d, CH_2)		
2	6.92 (d, arom), 6.68 (t, arom), 3.62 (s, CH_2), 3.54 (t, OCH_2)		
5	6.55 (s, arom), 3.83 (t, OCH_2), 3.62 (s, CH_2), 3.35 (t, OCH_2)		
$5 \cdot \text{NO}^+ \text{SbF}_6^-$	6.54 (s, arom), 3.93 (t, OCH_2), 3.76 (t, OCH_2),	1876	600
7	6.99 (s, arom), 4.39, 3.44 (2 x d, CH_2)	1923	563
8	7.17 (t, arom), 7.08 (d, arom), 3.87 (t, OCH_2)	1955	524

At 295 ± 1 K; The above reported spectral features of complexes $1 \cdot \text{NO}^+ \text{SbF}_6^-$, $2 \cdot \text{NO}^+ \text{SbF}_6^-$, and $5 \cdot \text{NO}^+ \text{SbF}_6^-$ are similar to those of complexes 7, 8, and 9, respectively; In CDCl_3 for ^1H NMR and FTIR, and in CHCl_3 for UVvis.

- 5 [00042] Independent structural evidence came from the complexation reactions between calixarenes 1 and 2 and commercially available $\text{NO}^+ \text{SbF}_6^-$ salt. (FIGURE 2 and 3) Specifically, the CDCl_3 solutions of compounds 1 and 2 were treated with $\text{NO}^+ \text{SbF}_6^-$ at about 295 K and the complexation induced changes in the UV-vis, FTIR and ^1H NMR spectra (as recorded). Under these conditions, the complexation process proved to be rather slow, however after about 20 hours no starting calixarenes 1,2 were observed and the
- 10 corresponding UV-vis, FTIR and ^1H NMR spectra exhibited features similar to those of nitrosonium complexes 7,8.

- [00043] Analogously, a nitrosonium complex of calixarene 5 was obtained. The UV-vis spectrum of the detected complex $5 \cdot \text{NO}^+ \text{SbF}_6^-$ (e.g., visible as dark blue) showed broad band
- 15 at about $\lambda_{\text{max}} \sim 600$ nm, and the FTIR spectrum exhibited characteristic arene- NO^+ stretching at about 1876 cm^{-1} . The ^1H NMR spectrum of $5 \cdot \text{NO}^+ \text{SbF}_6^-$ is different from complex 5 as well. For example, the methylene bridge CH_2 and both OCH_2 protons of complex 5 were seen as a singlet and two triplets, 1:1:1, at 3.62, 3.83 ($J = 7$ Hz), and 3.35 ppm ($J = 7$ Hz),

respectively. In complex $5\bullet\text{NO}^+\text{SbF}_6^-$, these were observed at 3.44, 3.93 ($J = 7.5$ Hz), and 3.76 ppm ($J = 7.5$ Hz), respectively.

[00044] The association constants for the above complexes were too high to be measured by the ^1H NMR technique. Even slight excess of $\text{NO}^+\text{SbF}_6^-$ results in the complete complex formation in CDCl_3 , and no free calixarenes **1**, **2**, and **5** were observed after equilibration.

The K_{ass} values $>10^6 \text{ M}^{-1}$ ($\Delta G^{295} > 8 \text{ kcal mol}^{-1}$) for the complexes were estimated and in agreement with published values in CH_2Cl_2 (e.g., see Rathore, R. et al. *Angew. Chem. Int. Ed.* 2000, vol. 39, pp. 2123-2127). While complexes $1\bullet\text{NO}^+\text{SbF}_6^-$ and $2\bullet\text{NO}^+\text{SbF}_6^-$ formed slowly, over about 20 hrs, it took only a few minutes to form complex $5\bullet\text{NO}^+\text{SbF}_6^-$.

Moreover, highly electrophilic NO^+ tended to further react with $5\bullet\text{NO}^+\text{SbF}_6^-$, and unidentified impurities were seen in the NMR spectrum after several minutes. Unlike complexes $1\bullet\text{NO}^+\text{SbF}_6^-$ and $2\bullet\text{NO}^+\text{SbF}_6^-$, which are chemically stable for weeks, complex $5\bullet\text{NO}^+\text{SbF}_6^-$ decomposed within a day (as observed by ^1H NMR).

[00045] Addition of H_2O or MeOH to the freshly prepared CHCl_3 solutions of complexes **7** and **8** and the nitrosonium complexes prepared from **1**, **2** and **5** and $\text{NO}^+\text{SbF}_6^-$, resulted in the complete complex dissociation and recovery of calixarenes **1**, **2** and **5** (as observed by preparative TLC, UVvis, ^1H NMR).

[00046] Spectral data for the reactions between compounds **1** and **2** and NO_2 , in the absence of SnCl_4 were re-observed. As excess NO_2 was passed through the solution of compound **1**, nitrosonium complex $[1\bullet\text{NO}^+]\text{NO}_3^-$ was clearly seen (UVvis, ^1H NMR), along with the mixture of nitration products. For compound **2**, no signals for nitrosonium complex $[2\bullet\text{NO}^+]\text{NO}_3^-$ were detected, because, while formed, these nitrosonium species quickly react with an excess NO_2 . Both reactions subsequently yield *p*-nitrated calixarenes.

[00047] As such, reactions between $\text{NO}_2/\text{N}_2\text{O}_4$ and *O*-alkylated calix[4]arenes proceed via the NO^+ encapsulation. In addition, calix[4]arene-nitrosonium complexes can be significantly stabilized by Lewis acids.

[00048] Host-Guest Dynamics of the Present Invention.

[00049] As evident from the ^1H NMR data, the NO^+ exchange in and out of the cavity is slow on the NMR time scale. For example, in the titration experiments between calixarenes 1 and 2 and $\text{NO}^+\text{SbF}_6^-$, both free and complexed species can be observed separately. This is typical for the host-guest complexes with high $K_{\text{ass}} > 10^6 \text{ M}^{-1}$ values. On the other hand, the NO^+ guest, with the van der Waals dimensions $< 2 \text{ \AA}$, freely migrates within the cavity at room temperatures (FIGURE 6). Indeed, the ^1H NMR spectra of complexes 7,8 possess the same symmetry as guest-free calixarenes 1,2, which in principle should be reduced upon complexation with nonsymmetrical NO^+ .

[00050] *Cone* calix[4]arene complex 7 should have a pinched, C_{2v} symmetrical conformation, since only two opposite, cofacial aromatic rings trap NO^+ . However, the observed at room temperature NMR spectrum exhibits a C_{4v} symmetry, indicating a fast (on the NMR time scale) exchange between two C_{2v} structures.

[00051] *1,3-Alternate* calix[4]arene complex 8 should exhibit a C_{2v} symmetry, with two different top and bottom halves of the skeleton. Instead, the observed symmetry is S_4 , with equal top and bottom halves.

[00052] In one aspect of the present invention, the complexation process is reversible, and the NO^+ guest can readily leave the calixarene cavity. For example, addition of H_2O to the freshly prepared CHCl_3 solutions of complexes 7,8 resulted in the complete dissociation and recovery of calixarenes 1,2. Complex 8 generally decolorized within seconds; complex 7 may take several minutes to decompose. Kinetics factors appears to be responsible for these observations, where *t*-Bu groups at the upper rim of the latter complex pose significant steric hindrances and protect the encapsulated NO^+ species from the entering H_2O . Such stability of the arene- NO^+ complex is without precedent.

[00053] In another aspect of the present invention, NO^+ guest can be transferred from one calixarene container to another (FIGURE 7). Calixarene 5 was specifically designed to

promote such transfer from the preformed complex **8**. Four additional, electron donating $\text{O}(\text{CH}_2)_5\text{CH}_3$ groups were introduced in *p*-positions to the initial set of $\text{O}(\text{CH}_2)_5\text{CH}_3$ groups. This makes cavity of compound **5** significantly more π -electron rich and dramatically increases its affinity towards positively charged NO^+ .

5 [00054] Examples of the exchange include obtaining complex **8** by treating calixarene **2** with 3 equivalents NO_2 and 1 equivalent SnCl_4 in CHCl_3 . Further, complex **8** and “empty” host **5** were mixed in an 1:1 ratio at 295 K in dry chloroform, and the UV-vis and ^1H NMR spectra were recorded over two hours. Due to the strong affinity of **2** towards NO^+ , the guest presence outside the cavity, in a bulk solution, was considered negligible; the only source of
10 NO^+ was complex **8**. Initially, the ^1H NMR spectrum exhibited only sets of signals for complex **8** and free calixarene **5**, and the corresponding UV-vis spectrum showed only the characteristic absorption for charge-transfer in complex **8**. Within minutes, however, the guest transfer was clearly detected. The band at λ_{max} of about 524 nm, assigned to complex **8**, systematically decreased and a new band at λ_{max} of about 600 nm, corresponding to new
15 complex **9**, appeared.

[00055] The nitrosonium transfer can be identified visually. For example, a purple solution of complex **8** in CHCl_3 turns blue upon addition of calixarene **5**. (FIGURE 8) When followed by ^1H NMR spectroscopy, the NO^+ exchange resulted in clean transformation of the spectra from mixture **8** + **5** to mixture **2** + **9**. (FIGURE 9) Note that the residual CHCl_3
20 signals in FIGURE 9 are marked with a dark circle and the decomposition products of compound **5** are marked with an asterisk. The methylene bridge CH_2 and OCH_2 protons of complex **8**, seen as a singlet and a triplet, 1:1, at 3.60 and 3.87 ppm ($J = 7.5$ Hz), may slowly decrease in intensity. Instead, two OCH_2 triplets at 3.93 and 3.76 ppm ($J = 7.5$ Hz) and the methylene bridge CH_2 singlet at 3.44 ppm appear and grow (assigned to complex **9**).
25 Complex **9** was confirmed in a series of independent experiments between **5** and $\text{NO}^+\text{SbF}_6^-$. Signals for “empty” calixarene **2** also appear, although slightly shifted due to the presence of SnCl_4 , and signals for complex **8** disappear. Within about an hour the nitrosonium transfer may be completed; both the ^1H NMR and UV-vis spectra exhibited only the signals of complex **9** and free calixarene **2**. No traces of initial complex **8** were detected.

[00056] This is a new example of a quantitative guest transfer between two different molecular containers. As such, molecular containers of the present invention serve as storage for information and processing, especially because they can be readily detected (e.g., by color changes) when the compounds are switched between two distinguishable states. In addition to switching from a free and entrapped state with the calixarene complexes of the present invention, the nitrosonium may be further transferred to another substrate (e.g., solid support, solid interface, etc. that accepts nitrosonium).

Functionalized Silica Gel of the Present Invention.

[00057] In the present invention, the interaction of calix[4]arene and a nitrosonium ion is: (a) reversible, (b) detectable (e.g., by color changes) and (c) effective for purifying NO_x compounds in the presence of other gases, such as H₂O, O₂, HCl, HBr, SO_x, and NH₃. In particular, these other vapors/gases are not involved in the same interactions/reactions with calixarenes. For example, neutral NO gas does not interact with calixarenes.

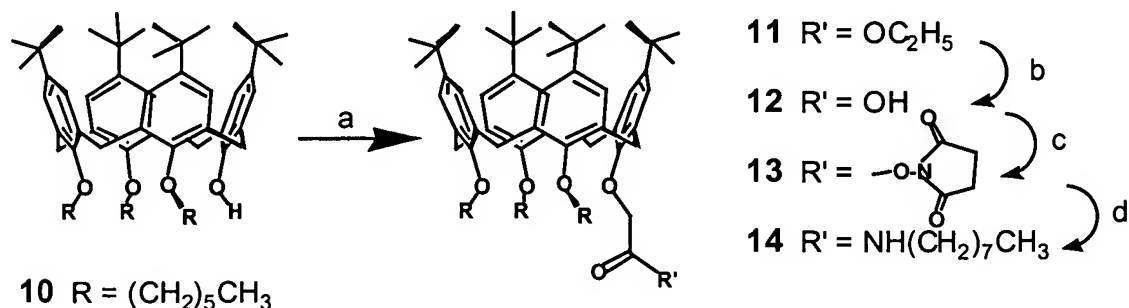
[00058] Current NO₂ sensors are generally electrochemical and monitor changes in potential upon exposure of metal surfaces to NO₂. In many cases however, other vapors - H₂O, O₂, HCl, HBr, SO_x, and NH₃ significantly influence detector selectivity and therefore its sensitivity. Optical sensors, generally based on color changes in a reaction between NO₂ and certain organic compounds, are more selective as the reactions are specific. However, reversibility of these sensing devices is not easy to achieve.

[00059] For application in any sensing technology, receptor molecules must not only be synthetically available, but also readily immobilizable on solid supports or surfaces. A wide variety of polymers and nanomaterials are commercially available. In one embodiment of the present invention, a solid support is functionalized with calixarene modules of the present invention (e.g., 3-aminopropylated silica gel). Molecular modeling of calix[4]arene fragments (of about 10 x 10 Å in dimension) attached to a solid support via the *lower* rim indicates the proper configuration of the *upper* rim to sufficiently respond to the presence of and to detect a gas analyte.

[00060] Calixarenes may be successfully attached to a surface, wherein tris-*O*-substituted calixarene **10**, prepared by selective alkylation of the parent calix[4]arene with *n*-hexylbromide and BaO/Ba(OH)₂²⁹ in DMF (69%), was further alkylated with ethyl bromoacetate to afford derivative compound **11** (Na₂CO₃, MeCN, 89%). (SCHEME 4)

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SCHEME 4



a) BrCH₂C(O)OC₂H₅, Na₂CO₃, MeCN, 80 degrees Centigrade, 12 h, yield of about 89 %; (b) KOH, THF-H₂O, 100 degrees Centigrade, 12 h, then aqueous HCl, yield of about or >95%; (c) *N*-hydroxy succinimide, DCC, DMAP, THF, room temperature, 12 hours, yield of about 83%; (d) CH₃(CH₂)₇-NH₂, Et₃N, THF, room temperature, 12 hours, yield of about 65%.

10 [00061] As in SCHEME 4, derivative compound **11** was hydrolyzed with KOH in a THF-H₂O mixture, resulting in calixarene acid **12** in a quantitative yield. Acid compound **12** was further activated with *N*-bromosuccinimide (DCC, DMAP, THF) to afford active ester compound **13** (yield of about 83%). Compound **13** readily reacts with amines. Thus, amide **14** was prepared from compound **13** and *n*-octylamine (Et₃N, THF, yield of about 65%) and

15 used for control experiments. Analogously, ester compound **13** was coupled to 3-aminopropyl-functionalized silica gel in THF in the presence of Et₃N to afford material **15**. (FIGURE 10) The presence of a calix[4]arene fragment in compound **15** was confirmed by the FTIR analysis in KBr disks: ν(CH) of about 2960 cm⁻¹ and ν(C=O) of about 1650 cm⁻¹ were recorded (similar to the stretching of model calixarene amide **14**: ν(CH) = 2964 cm⁻¹ and ν(C=O) = 1680 cm⁻¹). From the CHN analysis, about 17% calixarene loading was

20 achieved (there is steric bulkiness of the calixarene fragment).

[00062] In the NO₂ entrapment demonstration, a stream of the gas was passed through Pasteur pipettes, loaded with silica gel 15. (FIGURE 10) In one pipette, dry silica gel 15 was loaded, and the other contained 15 preliminary wetted with CHCl₃. With both silica gels, entrapment of a nitric oxide compound was instantly detected (e.g., color change, such as the gel turning dark purple) and indicated the NO⁺ complexation. When the material was wetted, the identification may be quicker and more complexation may occur (e.g., the color of the wetted material appeared to be deeper and lasted for hours). The dry material decolored within minutes. The FTIR spectrum, recorded in KBr disks, gave weak but reproducible stretch at ν of about 1920 cm⁻¹, indicating the presence arene-NO⁺ complexes. No detection was apparent for the pipette loaded with the starting solid support, e.g., 3-aminopropylated silica gel. For FIGURE 10, columns were prepared by (A) loading with starting aminopropyl functionalized silica gel, (B) loading with dry silica gel 15, (C) loading with 15 and flashed with CHCl₃ and then flashing all three columns with NO₂ (about 30 seconds) followed by photographing columns about 2-3 minutes later. For the present invention, calixarene cavities play a role in the described processes. Indeed, a solid support such as silica gel 15 is applicable for NO₂ detection and may even be used for purification of other nitrogen oxide compounds, especially NO.

Nitrosating Reagents of the Present Invention.

[00063] In addition to the previously described features of the present invention is the ability of calixarene-nitric oxide containing molecules. complex to act as nitrosating agents.

[00064] It is known, that reactions between NO⁺ generating agents (e.g., NOCl, N₂O₃, NO₂/N₂O₄, nitrosonium salts, etc.) and amides and short peptides proceed via *N*-nitrosation and yield biologically important nitroso-derivatives. Nitrosation of peptides may be used in analytical protocols, such as in protein sequencing, and occurs in biologic processes, when NO_x (e.g., as atmospheric pollutants) interacts with biological tissues and fluids. Here, the corresponding reaction mechanisms typically incorporate an electrophilic attack of NO⁺ on a nucleophilic oxygen or nitrogen of the substrate.

[00065] In yet another embodiment of the present invention, a sample such as *1,3-alternate* based nitrosonium complex **8** was mixed with AlkC(O)NHMe **16a-c** (Alk = *n*-Pr, *n*-Hex, *n*-Hept) in dry CHCl₃ and stirred at room temperature for several hours. (FIGURE 11) The corresponding *N*-nitrosoamides AlkC(O)N(NO)Me (**17a-c**) were formed in about 30-40% yield. Under the same conditions, the *cone* calixarene complex **7** reacted very slow, and only traces of the *N*-nitrosoamide products were detected by ¹H NMR spectroscopy. Likewise with water, *t*-Bu groups at the upper rim of complex **7** impose steric hindrances and protect the encapsulated NO⁺ species from the substrate. The rate limiting formation of the nitroso intermediates should take place within the calixarene cavity, prior to the NO⁺ dissociation. Once formed, these sterically bulky species leave the interior, and undergo further transformations in bulk solution.

[00066] These transformations provide additional structural evidence for the calixarene-NO⁺ complexes of the present invention and illustrate the utility of calixarenes as supramolecular/encapsulated nitrosating reagents (e.g., complexes as encapsulated reagents for nitrosation). As used herein, encapsulated reagents of the present invention are highly reactive species, reversibly entrapped within a host' cavity that may be released to the reaction mixture via one or more methods. The cavity offers protection from the bulk environment and thus controls the reaction rates. Reactions with encapsulated reagents may occur either within the cavity interior, or outside, upon release. Some of the more critical factors responsible for reagent release and for the reaction to occur are noncovalent forces holding the molecule-within-molecule complex together, such as temperature, solvent polarity and the substrate-cavity size-shape fit. Indeed, with deeper calixarenes, where the cavities could accommodate the reaction intermediates, supramolecular effects in these processes are more pronounced.

[00067] The present invention provides a new and improved NO_x sensing and fixation process that employs simple calix[4]arenes. With the present invention, calixarenes provide and transmit information about NO_x binding that can be readily detected (e.g., via visible light signals, electrochemical changes, spectroscopic changes), wherein the charge-transfer interactions allow the detection of various forms of nitrogen oxide compounds in the

presence of other gas mixtures, such as H_2O , O_2 , HCl , SO_x , NH_3 , NO , as well as chemical mixtures produced by chemical processing and manufacturing. The calixarenes and complexes of the present invention may be immobilized, in solution or attached to a ligand. The resulting calixarene-nitrogen oxide compound complexes may be used as vehicles for
5 nitrosonium transfer and encapsulated nitrosating reagents. NO_2/NO_x sensing molecules and as storing materials.

[00068] Additional objects, advantages and novel features of the invention as set forth in the description, will be apparent to one skilled in the art after reading the foregoing detailed description or may be learned by practice of the invention. The objects and advantages of the
10 invention may be realized and attained by means of the instruments and combinations particularly pointed out here.